PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT EXAMINING OPERATION

Applicant(s): Glen H. ERIKSON et al.

Serial No: 09/911,047 Group Art Unit: 1634

Filed: July 23, 2001 Examiner: Betty J. Forman

Att. Docket No.: E1047/20060 Confirmation No.: 3230

For: HOMOGENEOUS ASSAY OF BIOPOLYMER BINDING BY MEANS OF MULTIPLE MEASUREMENTS UNDER VARIED CONDITIONS

DECLARATION UNDER 37 CFR 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Glen Erikson, Jasmine Daksis and Pierre Picard, hereby declare that:

1. We understand from patent counsel for the assignee of the above noted application, Ingeneus Corp., that we can antedate U.S. Patent No. 6,391,624 and U.S. Patent Application Publication No. 2002/0094531 by showing a reduction to practice of the invention prior to the earliest effective filing dates of the references, which are respectively June 3, 1999 and June 14, 1999.

2. A reduction to practice of an embodiment of the invention claimed in the above-identified application occurred in Canada prior to June 3, 1999 and subsequent to December 8, 1993 (which we understand from patent counsel was the effective date of Section 331 of the North American Free Trade Agreement Act). Evidence of such embodiment is shown by the reproductions of several pages of laboratory notebook entries attached as Exhibit A. The reproductions are redacted to remove references to specific dates, as Applicants choose to merely allege reduction to practice prior to the effective filing dates of the references, rather than reveal the actual dates of the reduction to practice. See MPEP § 715.07 II

3. Exhibit A comprises four graphs of fluorescent intensity against fluorescent wavelength. Each of the graphs comprises a composite of the following three spectra: (1) a spectrum taken from a reaction mixture in the absence of applied voltage (0V), (2) a spectrum taken after applying a voltage of 1V for 3 seconds; and (3) a spectrum taken after applying a

voltage of 1V for 15 seconds. The graphs were based on experiments similar in protocol to Example 5 of the application, and consequently resemble Figs. 5A and 5B of the application in format. The hybridization reaction mixtures contained target dsDNA, PNA probes, buffer solution, and the DNA intercalator YOYO-1, in which the probes were a perfect match or a 1 bp, 2 bp or 3 bp mismatch to a sequence present in the target DNA. The reaction mixtures were incubated at 95°C for 10 minutes to allow denaturation, placed in a quartz cuvette maintained at 65°C, and then irradiated with an argon ion laser beam having a wavelength of 488 nm and monitored for fluorescent emission.

4. Fluorescent intensities were plotted as a function of wavelength for each sample analyzed. DNA:PNA hybrids consisting of perfectly complementary sequences were most resistant to the destabilizing effect of the applied voltage (Notebook Page 64). The reduction in fluorescent intensity as a function of applied voltage was greater for a 1 bp mismatched DNA:PNA hybrid (Notebook Page 65), still greater for a 2 bp mismatched DNA:PNA hybrid (Notebook Page 66), and greatest for a 3 bp mismatched DNA:PNA hybrid (Notebook Page 67). Thus, as the degree of sequence complementarity between the probe and the target decreased, the level of fluorescent intensity diminished dramatically in response to applied voltage, providing a highly reliable and accurate means to differentiate between perfectly matched sequences and those containing 1 bp, 2 bp or 3 bp mutations, regardless of the relative level of fluorescent emission detected prior to the application of voltage.

5. Thus, the experimental results of Exhibit A show an embodiment of a method for assaying sequence-specific hybridization, said method comprising: (1) providing a target comprising at least one target biopolymer sequence (i.e., DNA); (2) providing a probe comprising at least one probe biopolymer sequence (i.e., PNA); (3) adding said probe and said target to a binding medium (i.e., buffer solution) to provide a test sample; (4) applying a first stimulus (i.e., irradiation with a laser) to said test sample to provide a first stimulated test sample; (5) detecting a first signal (i.e., pre-electrification fluorescent intensity emission) from said first stimulated test sample, wherein said first signal is correlated with a binding affinity between said probe and said target; (6) applying a second stimulus (applied voltage) to said first stimulated

test sample to provide a second stimulated test sample; (7) detecting a second signal (i.e., post-electrification fluorescent intensity emission) from said second stimulated test sample, wherein said second signal is correlated with said binding affinity between said probe and said target; and (8) comparing said first signal and said second signal to accomplish said assaying (i.e., comparing pre-electrification intensity with post-electrification intensity to identify whether the probe and target are perfectly complementary, or mismatched by 1, 2 or 3 base pairs); wherein: (a) at least one label (i.e., YOYO-1) is provided in said test sample, (b) said first stimulus, said second stimulus, said first signal and said second signal are photonic (i.e., laser) or electronic (i.e., applied voltage), (c) at least one of said first stimulus and said second stimulus is photonic, and (d) when said first stimulus and said second stimulus are photonic, an intermediate electronic stimulus is applied to said test sample after said first stimulus and before said second stimulus. The experimental results were sufficient to prove that the claimed invention was suitable for its intended purpose.

6. Accordingly, we achieved an actual reduction to practice of embodiments of the claimed invention prior to June 3, 1999.

We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 11 August, 2004

Glen Erikson

Date: 10 August 2004

Jasmine Daksis

Date: _____

Pierre Picard

Attachment: Exhibit A

test sample to provide a second stimulated test sample; (7) detecting a second signal (i.e., post-electrification fluorescent intensity emission) from said second stimulated test sample, wherein said second signal is correlated with said binding affinity between said probe and said target; and (8) comparing said first signal and said second signal to accomplish said assaying (i.e., comparing pre-electrification intensity with post-electrification intensity to identify whether the probe and target are perfectly complementary, or mismatched by 1, 2 or 3 base pairs); wherein: (a) at least one label (i.e., YOYO-1) is provided in said test sample, (b) said first stimulus, said second stimulus, said first signal and said second signal are photonic (i.e., laser) or electronic (i.e., applied voltage), (c) at least one of said first stimulus and said second stimulus is photonic, and (d) when said first stimulus and said second stimulus are photonic, an intermediate electronic stimulus is applied to said test sample after said first stimulus and before said second stimulus. The experimental results were sufficient to prove that the claimed invention was suitable for its intended purpose.

6. Accordingly, we achieved an actual reduction to practice of embodiments of the claimed invention prior to June 3, 1999.

We further declare that all statements made herein of our own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

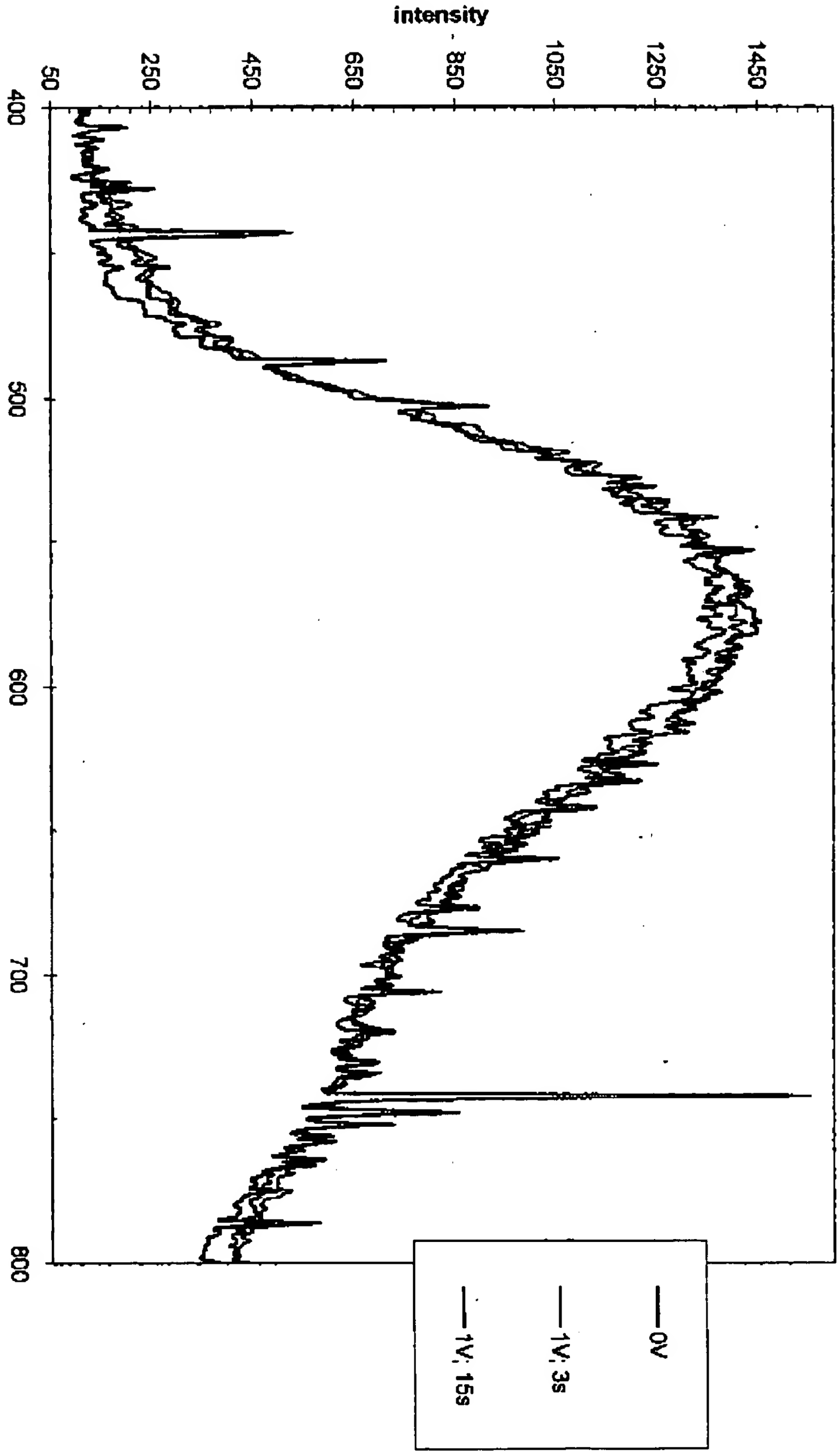
Date:_____ Glen Erikson

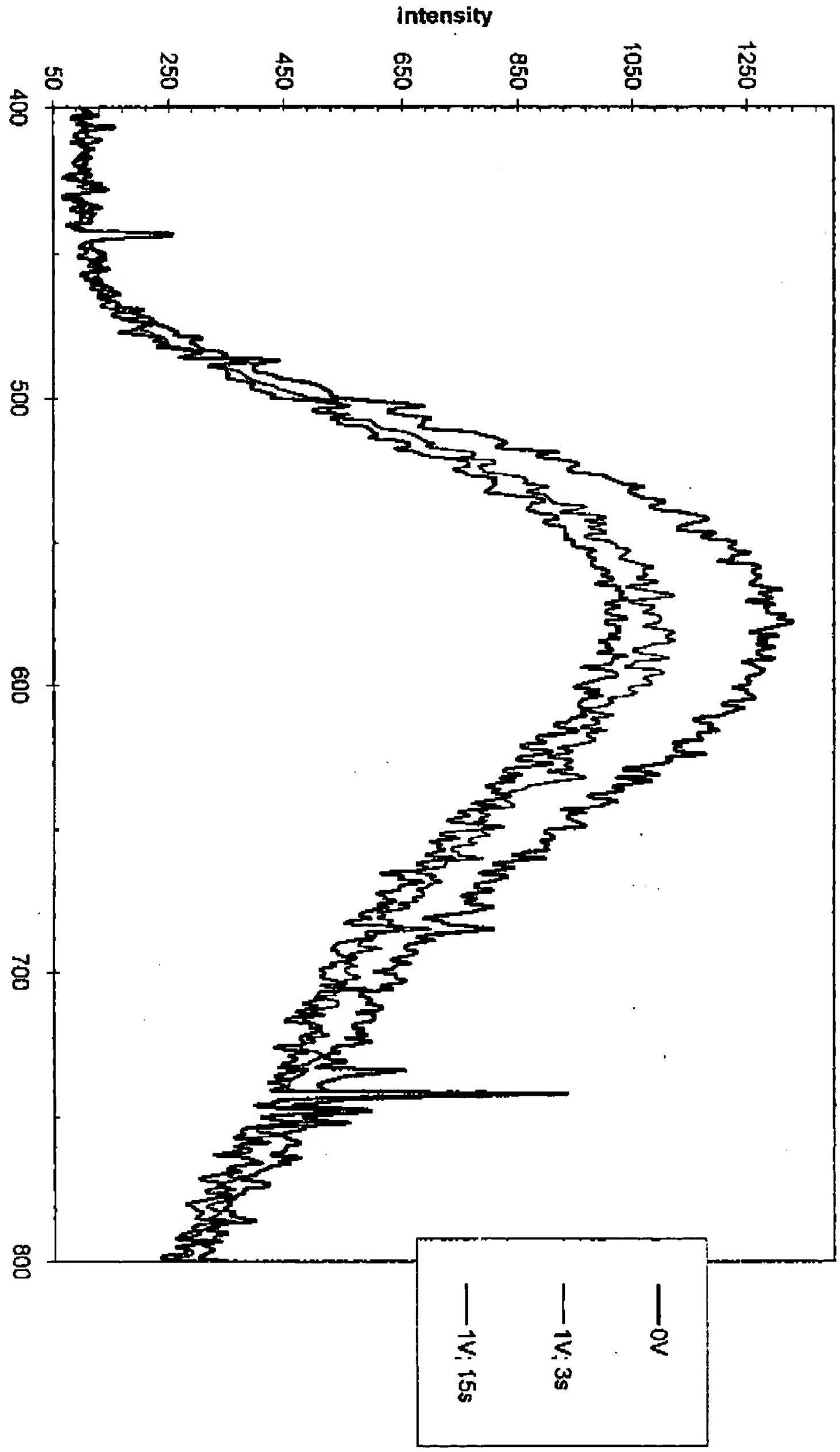
Date:_____ Jasmine Daksis

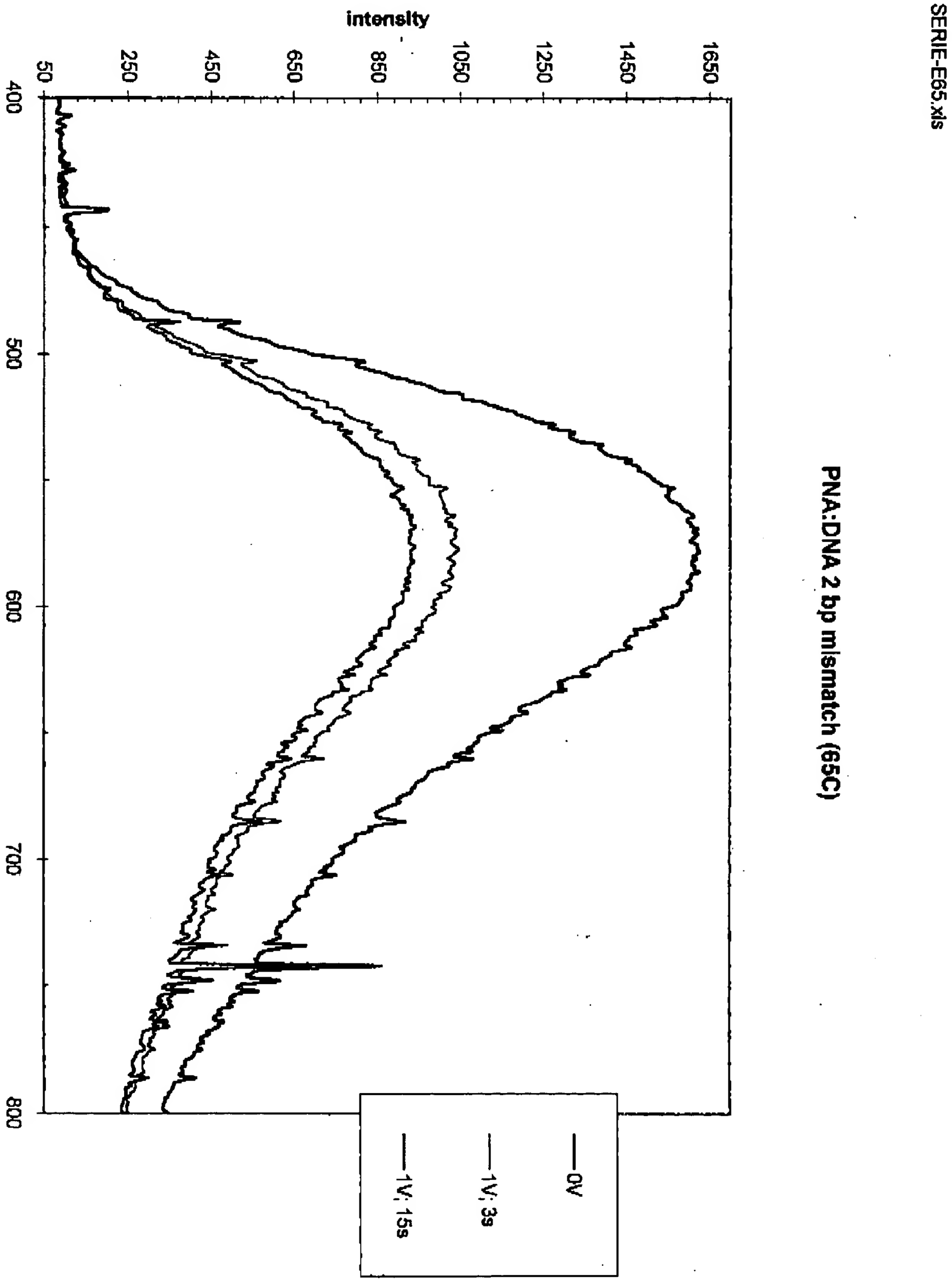
Date: 23/8/04 Pierre Picard

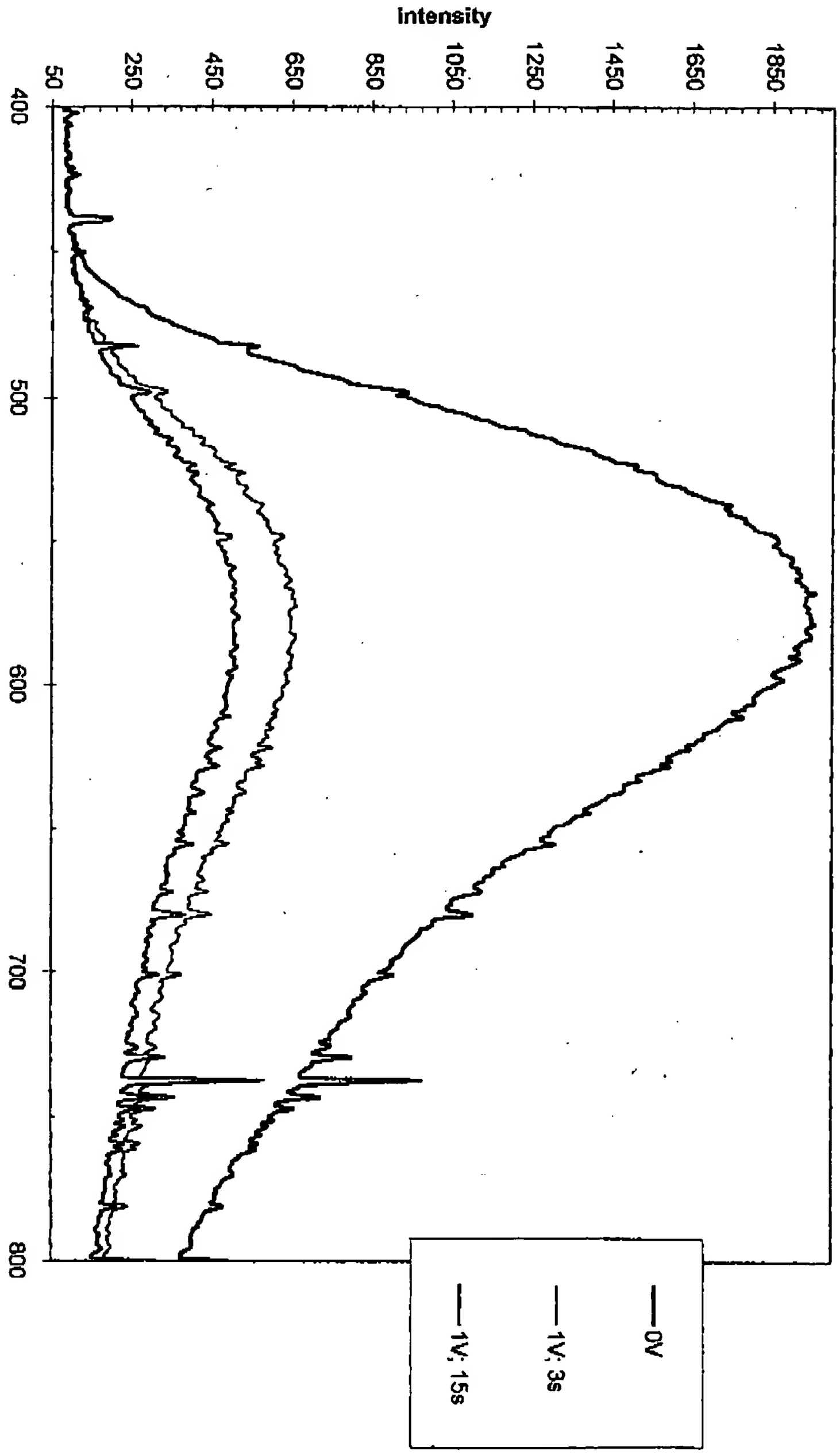
Attachment: Exhibit A

64


intensity
50
250
450
650
850
1050
1250
1450
400
500
600
700
800
wavelenght
PNA:DNA perfect match (65C)
0V
1V; 3s
1V; 15s
2, SERIE-E65.xls


3, SERIE-E65.xls
PNA:DNA 1 bp mismatch (65C)
Intensity
50
250
450
650
850
1050
1250
400
500
600
700
800
wavelenght
1V; 15s
1V; 3s
0V


4, SERIE-E65.xls
PNA:DNA 2 bp mismatch (65C)
intensity
50
250
450
650
850
1050
1250
1450
1650
400
500
600
700
800
wavelenght
0V
1V; 3s
1V; 15s



5, SERIE-E65.xls

PNA:DNA 3 bp mismatch (25C)